Ecological and evolutionary factors determlning the regional variations of echolocation call frequency in the horseshoe bat on Okinawa-jima Island

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学位記番号	生博第１８１号
学位授与年月日	平成２２年３月２５日
学位授与の要件	学位規程第４条第１項該当
研究科，専攻	東北大学大学院生命科学研究科 （博士課程）生態システム生命科学専攻
論文題目	Ecological and evolutionary factors determining the regional variations of echolocation call frequency in the horseshoe bat on Okinawa-jima Island.（沖縄島におけるコキクガシラコウモリのエコーロケーションコールの地域間変異に関する生態学的及び進化学的要因について）
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論文内容の要旨

Bats use echolocation call to orient in space, and often for the detection, localization and classification of prey, and depend highly on audible systems to get any information of surrounding night environment. Evolution of echolocation calls are directly related to the diversification in bats taxa. Bats in the families Rhinolophidae and Hipposideridae produce echolocation pulses dominated by a constant frequency (CF) component. The previous study indicated that CF transmission is a function of both genes and cultural factors with some other effects such as body conditions. The intra-specific differences of CF in echolocation call are associated with variation in food/resource use and mating signals, communication, which could lead to adaptive divergence and speciation. However, the origin and meaning of CF variation within rhinolophid bats is not well understood despite an increasing number of allopatric and sympatric examples being documented.

Differences in echolocation call frequency among these groups have been explained by several competing hypotheses involving factors relating to insect size, detection distance and foraging habitat; social transfer between mother and offspring or among colony members and simple drift associated with changes in body size. In addition to these factors, social selection in which an individual's fitness is determined in part by the phenotype of its social partners, might be an important factor causing CF divergence. Since individuals should be most receptive to auditory signals from conspeciefics that are closest to their own call frequency which elicit the greatest auditory neurological response, the selection of social partners based on shared phenotypic attributes results in difference of inter-population difference.

The situation of the endangered species *Rhinolophus cornutus pumilus* (Rhinolophidae) on Okinawa-jima Island in the Japanese Ryukyu Archipelago provided a unique opportunity to examine one part of this complex problem in a group at an earlier stage of evolution. The island is elongated north–south over 106 km, with a narrow constriction in the middle region with over 28km forming a range of hilly forest area (Fig. 1). I analyzed variation in CF of the echolocation call from 11 caves scattered over the Okinawa-jima Island, and found clear acoustic

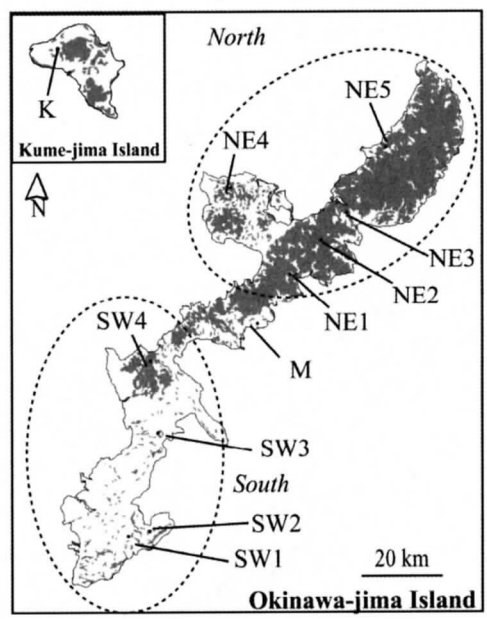

Fig. 1. Map of Okinawa-jima and Kume-jima Islands showing locations of the 11 cave populations, where data for *Rhinolophus pumilus* were obtained. Grey shading indicate zones of forest area.

differences (5–8 kHz) observed between the north and south regions with some differences within regions (Fig. 2). The landscape differs between north (predominantly forested) and south (predominantly agricultural and urban) Okinawa, indicating that the availability of different-sized or types of prey and/or the distance at which bats forage from vegetation might be different between the north and south. However, there is no obvious environmental factor evident from this study that could have been the basis for selection.

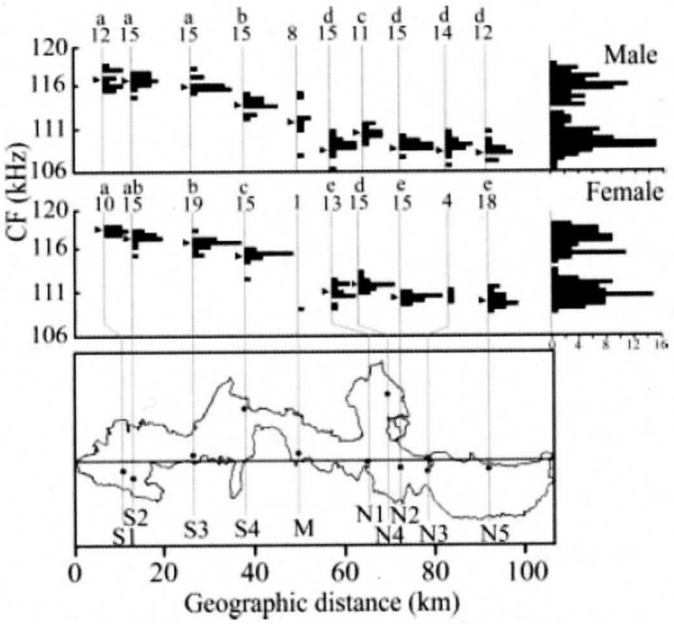

Fig. 2 Variation in the constant frequency (CF) component of echo-location calls among Okinawa-jima colonies for males and females separately. Samples sharing the same letters showed no significant differences in CF. Females sampled from M and N3 were excluded from analyses of CF because of small sample size.

A partial mitochondrial DNA D-loop sequences and genotypes from six microsatellite loci were obtained from the 11 colonies. The acoustic differences observed between the north and south regions have been maintained despite evidence of sufficient nuclear gene flow across the middle of the island (e.g. Fig. 3). Significant subdivision of maternally inherited D-loop haplotypes suggested a limitation of movement of females between regions, but not within the regions, and was evidence of female philopatry (e.g., Fig. 3).

With the results in molecular genetics, I proposed a 'maternal transmission' hypothesis whereby the difference in the constant frequency (CF) component between the regions is maintained by mother–offspring transmission of CF, the restricted dispersal of females between regions and small effective population size. However, to confirm the hypotheses, I should consider the effect of social selection on CF divergence and examine whether maternal transmission and culture drift alone can cause

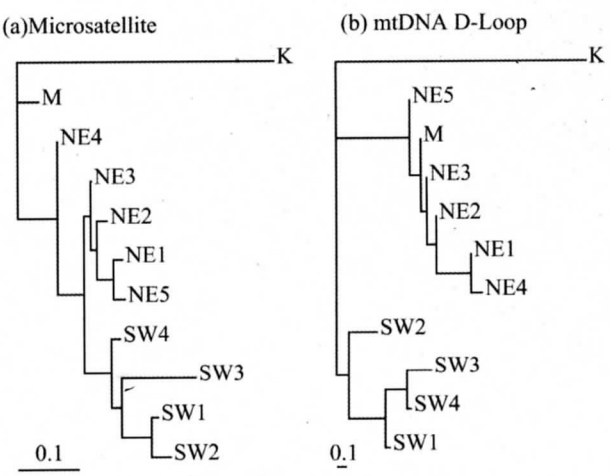

Fig. 3 Neighbour-joining trees from genetic distances for 10 populations of *R. pumilus* on Okinawa-jima and one population on Kume-jima Island (K). (a) for six microsatellite loci, and (b) for mtDNA D-Loop region sequences.

observed differences in CF in Okinawa Jima.

To estimate the factors causing maintenance of CF differences, I conducted individual-based simulations in which CF of individuals transmitted into the next generation through additive polygenes and maternally inheritance. In the simulations, I also consider social selection as a factor affecting CF variations. If CF variations are affected by polygenetic variations, gene flows between the regions would reduce the CF divergence between the regions. On the other hand, maternally inheritance of CF with female philopatry and social selection might promote the maintenance of CF differences between the regions. Under the observed levels of gene flows within the island, we estimate the degree of social selection and accuracy of maternally inheritance of CF that lead to the observed differences between the regions (5-8Hz) and within colony variation of CF. I also examined whether CF difference between the regions could be originated parapatrically within the island through maternally inheritance of CF with female philopatry and social selection.

The results of the individual-based simulations showed that when moderate social selection as well as the maternal transmission with some error were assumed, the regional CF difference could have been maintained with the sufficient degree of gene flows during short periods less than 3000 years. This suggests that both the maternal transmission and social selection are necessary factors for the maintenance of parapatric CF variation of *R. c. pumius* on the Island. The model results also showed that the variation in CF between the regions could not be produced parapatrically from the homogeneous CF variation within the island. Thus, CF might diverge between ancestral isolated populations by genetic or cultural drift, and after the secondary contact, CF differences have been maintained under the levels of gene flows by a process of mother-offspring transmission with the limited dispersal of females between regions and social selection. The results also suggested that the acoustic differences in *R. c. pumilus* observed between the regions on the island are not stable, and would be disappear if the present levels of gene flows will be kept. The present thesis focused mainly on the CF differences of *R. c. pumilus* on Okinawa-jima Island, but the results also suggest that maternal transmission and social selection play important roles in CF variations among populations within species and between species in Rhinolophus groups.

論文審査結果の要旨

本研究は、沖縄島のコキグラシラコウモリにおけるエコロケーションコールの周波数の地理的変異の維持機構について、分子生態学的およびシミュレーションモデルによって解明した研究である。コウモリのエコロケーションコールの変異は、コウモリ間のコミュニケーション、交配、餌獲得などに影響すると考えられ、生態学的および進化学的に重要な性質である。本研究によって以下のことが明らかになった。(1)沖縄島の南部と北部の間で、エコロケーションコールの CF 部と呼ばれる周波数が 5-8kHz の違いがあることが明らかになった。(2)マイクロサテライトによる解析から、北部と南部の間では、遺伝子流動が十分にあることが示された。(3) ミトコンドリア DNA である D-Loop から推定された Fst およびハプロタイプ頻度の解析からは、遺伝子流動が南北間で妨げられていることが示され、このことから、南北間では、雌の分散が制限されていることが推定された。(4) 以上の結果と、CF 値は、母親から子供に伝わるという報告、5-8kHz の違いは餌獲得能力に差がでないという解析をもとに、北部と南部の間で、雄の分散が生じているなかで周波数の違いが維持される条件をシミュレーションモデルを用いて解析した。(5) シミュレーション解析の結果、周波数の母子伝搬と雌の移動分散の制限だけでは、5-8kHz の周波数の変異を長期にわたって維持することが困難であることが示された。(6)同じコロニーの中で、異なる周波数を示す個体の適応度が低下するという社会選択を仮定して、同様のシミュレーション解析を行った結果、周波数の母子伝搬の正確さと社会選択の強さの適当な組み合わせで周波数の違いが維持されることが明らかになった。(7)これらのことから、沖縄島の観察されるコキグラシラコウモリにおけるエコロケーションコールの周波数の地理的変異には、周波数の母子伝搬と雌の移動の制限、さらに社会選択が関わっていることが示された。

これらの結果は、地理的変異と多様性の維持の解明に重要な示唆をあたえるものであり、吉野元氏が自立して研究活動を行うに必要な高度の研究能力と学識を有することを示している。したがって，吉野元氏提出の論文は，博士（生命科学）の博士論文として合格と認める。